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PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

R. CHANEY et al. | Art Unit No.: 1638

Application No: 09/437,607 | Examiner: M. Ibrahim

Filed: November 10, 1999 | Atty. Dkt. No.: 108172-00037

For: RECOVERING METALS FROM SOIL

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner of Patent
Washington, D.C. 20231

Sir:

I, Yin-Ming Li, of Potomac, MD, declare and state that:

I am one of the inventors in the above-identified application and I have read and understood the Office Action of August 13, 2002 and the rejections cited against the pending claims.

My comments with respect to the claimed invention are as follows:

1) The plant species used by Raskin et al. (U.S. Patent No. 5,785,735) are not natural metal-hyperaccumulator plants as used in the claimed invention, and as would be recognized by those of ordinary skill in the art.

2) The metal phytoremediation patents awarded to Raskin et al. (*1-3*), including U.S. Patent No. 5,785,735 (*2*), utilize plant species that are not tolerant to high levels of heavy metals and that do not hyperaccumulate metals from most soils.

These species are commonly used as food crops. Th y include *Brassica juncea* (L.) Czern. (Indian mustard), *B. carinata* Braun (Ethopian mustard), *B. napus* (L.) (rapeseed), *B. oleracea* (L.) (cole crops), *B. nigra* (L.) Koch (black mustard), *B. campestris* (L.) (turnip rape), *Raphanus sativus* (L.) (radish), and *Sinapis alba* (L.) (white mustard).

3) Indeed, the Raskin et al. patents explicitly exclude wild hyperaccumulator species, such as *Alyssum murale*, which are endemic to naturally-occurring high metal soils and have evolved tolerance to the metals contained in the soils. Moreover, the Raskin et al. patents focus exclusively on members of *Brassicaceae* and thus by definition exclude the remaining non-*Brassicaceae* natural hyperaccumulator species identified in the claimed invention.

4) While Raskin et al. may have achieved high levels of metals in crop species, this has been possible only through a variety of elaborate and expensive supplemental treatments. For example, in the 1994 patent (*1*), Raskin et al. describes screening seedlings by growing them on a mixture of sand and perlite and then adding soluble metal salts. Sand and perlite have much lower metal sorbing capacity than typical soils. Most soils contain clay and organic matter which strongly adsorb metals, greatly reducing their availability to crop plants. In addition, Raskin et al. excluded the plant nutrients phosphate and sulfate from the growing medium, because these compounds bind strongly to metals and reduce their availability to plants. In a paper describing this work (*4*), it is stated that lead (Pb) treatment for 14 days inhibited shoot

growth by at least 50% compared to the controls. It is noted that even though short-term xperiments like this one may be considered inad quate for fully evaluating plant metal tolerance, it is clear that these species are not metal (i.e., Pb) tolerant.

5) In a 1997 review by Huang et al. (*5*) of Pb phytoextraction research carried out by Huang et al. and Raskin et al., it is stated that none of the high-biomass crop species tested could accumulate greater than 0.1% Pb in the shoot (the criterion for hyperaccumulation) when grown on Pb-contaminated soils without soil amendments. In the 1998 patent (U.S. Patent No. 5,785,735) (*2*) and 2000 patent (*3*), Raskin et al. describes achieving high metal concentration in crop shoots by adding synthetic chelators to soils. The chelators are chemicals that strongly bind to metal ions, making them mobile in the soil environment. In some cases, the metal-chelate complex is taken up by plants, leading rapidly to plant death due to toxic effects from both the metal and the chelate. Thus, this method requires that the plant be grown to full size before the chelate is added to the soil.

6) The 1998 patent (U.S. Patent No. 5,785,735) (*2*) and 2000 patent (*3*) also describe the use of soil acidification to increase the availability of soil metals. In a paper describing the application of this technique to *Brassica juncea* grown on cadmium-contaminated soil, Zaurov et al. (*6*) reports that decreasing soil pH to 5.5 (from the normal 6.5) resulted in increased cadmium concentration in plant shoots, but both plant growth and total cadmium uptake were greatly reduced at the lower pH.

7) Thus, it can be seen that increasing metal availability in soil is

counterproductive when the plant being us d for phytoextraction does not have th metal tolerance found in natural metal-hyperaccumulator plants.

8) In contrast to the work by Raskin et al., in the claimed invention, the response of natural-hyperaccumulator plants including various species of *Alyssum, Thlaspi and Berkheya* to soil pH is unique. Our work with *Alyssum, Thlaspi and Berkheya* has yielded, to our knowledge, the first report of shoot metal increasing with increasing soil pH.

9) In the few previous reports on the effects of soil pH on metal hyperaccumulation, only the usual response of more metal uptake at lower pH has been seen. Robinson et al. (*7*), studying a naturally occurring population of the Ni-hyperaccumulator *Alyssum bertolonii* on a serpentine soil in Italy, found that liming to pH 7.94 reduced Ni concentration in *A. bertolonii*, in comparison with an unlimed control at pH 7.37. Two other reports, one on the Zn hyperaccumulator *Thlaspi caerulescens* (*8*) and one on the Ni hyperaccumulator *Berkheya coddii* (*9*) found that shoot metal concentration increased as soil pH was lowered to between 5.5 and 6.0 by sulfur application.

10) Likewise, among non-hyperaccumulator plants, the universal response is increased shoot metal with decreasing soil pH. Zaurov et al. (*10*), for example, in a paper describing the effect of altering soil pH on uptake of cadmium by *Brassica juncea* grown on cadmium-contaminated soil, reported that decreasing soil pH to 5.5 (from the normal 6.5) resulted in increased cadmium concentration in plant shoots.

11) Yet, in field experiments conducted on nickel-contaminated soil in Ontario, Canada, we hav grown *Alyssum* in the same plots with the agricultural crops corn, soybeans, oats, and radish. When soil pH was raised by liming, nickel concentration in *Alyssum* was raised, while that of the crop species was lowered (unpublished results). We have done additional studies in the greenhouse and laboratory with various species of *Alyssum, Thlaspi* and *Berkheya* that have confirmed the pH effect described in the claimed invention.

12) Clearly, the plant species used by Raskin et al. in the U.S. Patent No. 5,785,735 are not natural metal-hyperaccumulator plants as used in the claimed invention. That is, the plants of Raskin et al. do not yield increases in shoot metal with increases in soil pH, as in the claimed invention.

13) I hereby declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the application or any patent issued thereon.

02/13/03
Date

Yin-Ming Li per J.D.N (by permission)
Yin-Ming Li

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